SCIENTIFIC ABSTRACT

Over 50,000 new cases of bladder cancer are diagnosed annually in the U.S., and 10,000 patients die yearly from the disease. Transitional cell carcinoma (TCC) is the predominant type of urinary tract cancer, comprising >90% of all bladder cancers. At the time of diagnosis approximately 80% are considered to be superficial, which is defined as tumor confined to the bladder mucosa (Tis, Ta and T1). The remaining 20% of cases are considered to be invasive, which is defined as tumor invading the muscle layer of the bladder or beyond (stages T2-T4); or bladder cancer metastatic to lymph nodes or distant sites. The natural history of this disease is predictable, involving tumor recurrence in >50% of patients treated by transurethral endoscopic resection of the bladder tumor (TURBT). In approximately 10% of cases, tumor recurrence is associated with progressive tumor growth and invasion into the muscle layer or beyond. Despite aggressive treatment programs, approximately 50% of patients with invasive tumors die of bladder cancer within 5 years.

Mutation or loss of expression of the retinoblastoma (RB) gene product can occur as an early or later genetic event in the development of bladder cancer. RB alteration occurs in approximately 10-50% of patients with superficial bladder cancers, and in 30-80% of invasive bladder cancers. Bladder cancers with RB-alterations are associated with particularly poor prognosis. Restoration of RB function by gene transfer into RB-altered bladder cancer cells in culture and in animal models has resulted in suppression of tumor growth.

The human RB gene is a tumor suppressor gene involved in the control of cell proliferation. In the normal cell cycle, hypophosphorylated RB arrests cell proliferation in the G_0 and G_1 phase of the cell cycle by inhibiting the function of growth promoter elements, such as E2F. Passage through the G_1 checkpoint commits cells to DNA synthesis and cell division. Inappropriate passage through this checkpoint may cause cells to synthesize new DNA in S phase from damaged templates before scheduled DNA repair can be completed in G_1 or to miss signals to exit the cell cycle for differentiation or cell death. RB, therefore, functions as a gatekeeper in the normal cell cycle by suppressing uncontrolled cellular proliferation. The reintroduction and expression of wild-type RB into RB-altered tumor cells has been shown to suppress tumor growth in both *in vitro* and *in vivo* models.

The ACNRB construct is a recombinant, replication-defective adenovirus derived from adenovirus serotype 5 (Ad5), subgroup C. The adenoviral E1a, E1b and protein IX coding sequences are deleted and replaced with the RB expression cassette. The deleted E1 region is necessary for viral replication. The virus is additionally deleted for 1.9 kb of DNA sequence in Early Region 3 and 1.4 kb of DNA sequence in Early Region 4. The RB expression cassette contains the human cytomegalovirus immediate early promoter-enhancer, the adenovirus type 2 tripartite leader sequence and a cDNA sequence encoding wild-type $p110^{RB}$ protein. The human cytomegalovirus immediate early promoter-enhancer directs robust gene expression and the adenovirus type 2 tripartite leader sequence enhances translational efficiency. Polyadenylation is regulated by the E1b and pIX polyadenylation signal contained within the adenoviral genome. All other regulatory elements within ACNRB are endogenous to Ad5. The recombinant adenovirus is similar to other adenoviral vectors reviewed by the RAC and the FDA except that it contains additional deletion of the E4 and pIX coding sequence. The deletion in the pIX coding sequence is expected to reduce the frequency of replication competent adenovirus arising during virus production by reducing the sequence identity with the E1 sequences of the 293 production cell line.

The clinical study will evaluate the safety, biological efficacy, including efficiency and stability of gene transfer, and the effect of dose of intravesical instillation of ACNRB in patients with superficial and muscle-invasive bladder cancer. The study design is an open-label, non-randomized, single-dose, dose escalation Phase IA clinical trial anticipated to involve a maximum of 24 patients. ACNRB will be administered intravesically via a catheter in escalating doses to successive cohorts of patients until the maximum tolerated dose is determined. Study patients will have failed at least one course of standard therapy and have evidence of RB alteration in their bladder tumors.